Expanding the Vision of Environmental Health at UNC-CH

In a town that devotees call the "Southern Part of Heaven," the University of North Carolina at Chapel Hill (UNC-CH) is known nationally for its schools of public health, medicine, and pharmacy. These schools are physically just across the street from one another, but they can seem worlds away if their knowledge and resources aren't shared. To bring these researchers together, the Center for Environmental Health and Susceptibility (CEHS) was funded with a $3.78 million, four-year grant from the NIEHS to become one of 22 Environmental Health Sciences Centers. "We have an unusual mix of basic, clinical, and population scientists," says CEHS director James Swenberg, a UNC-CH professor of environmental sciences and engineering. "The center's goal is to, by bringing these groups together, expand our vision of environmental health research."

Center researchers work to understand the fundamental processes that contribute to chemical toxicity and to combine this knowledge with epidemiology to reduce environmental disease. Increasingly, work at the center focuses on understanding how environmental and genetic determinants of disease work together in populations. And whereas researchers have in the past focused largely on average susceptibility when looking at the distribution of disease among a particular population, CEHS scientists are among those now looking at populations with greater- or lesser-than-average disease susceptibility, Swenberg says. Furthermore, they are trying to help people understand what role their genetic makeup plays in susceptibility.

That expanded vision can be seen in the center's recent growth, in April 2004, from three research cores to five. The center also includes four facility cores to offer services such as high-throughput genetic analysis, provision of a variety of biomarkers, and expertise on statistical design and analysis. And the Community Outreach and Education Program (COEP) brings what researchers are learning to the people of North Carolina and beyond.

Capitalizing on an Investment in Genomics

The genomics revolution has contributed to the fast growth of the CEHS. In the past few years, UNC-CH has made a large investment in genome science, including creating a Department of Genetics in its School of Medicine and building a proteomics facility. Partly because of the ability to examine a greater number of samples faster than ever before, researchers are no longer looking at single-nucleotide polymorphisms in isolation. "We're learning that it's much more complex than that," Swenberg says. "Now researchers are moving toward looking at haplotypes—how groups of genes are interacting to give greater or lesser susceptibility." One of the CEHS's new research cores, the Transomics Research Core, directed by genetics researcher David Threadgill, works to capitalize on these investments by applying new technologies such as genomics, proteomics, and metabolomics to the understanding of environmental health risks.

Recent work by geneticist Charles Perou is an example of this trend. Using microarrays, which produce fluorescent images that allow researchers to monitor the expression of 20,000 genes at once, Perou is able to look for patterns of gene expression in thousands of tumors. He has shown that human breast tumors arise from at least two distinct types of cells—basal and luminal epithelial cells—and that basal cell carcinomas are less responsive to chemotherapy.

Perou has found that the basal subtype does not respond as well to p53, the "guardian" protein that induces programmed cell death in response to DNA damage. This difference may partly explain the basal subtype's resistance to treatment such as chemotherapy, which involves induction of apoptosis. Perou is also working with other investigators in the Toxicogenomics Research Consortium, funded by the NIEHS, to use microarrays to study patterns of cellular response to environmental toxicants such as those in car exhaust and cigarette smoke.

Closely related to the work of Transomics Research Core is that of the Genetic Susceptibility Research Core, directed by William Kaufmann, a professor of pathology and laboratory medicine. This core seeks to find out how variations in DNA from individual to individual affect the risk of disease caused by environmental exposure. The core's work focuses on how gene–environment interactions affect DNA repair and genetic susceptibility to diseases such as cancer, asthma, and heart disease.

Using Chinese hamster cell lines, environmental scientist Jun Nakamura, collaborating with Yoshiko Kubota of Japan's Akita Medical University, found that only one out of several identified polymorphisms in *XRCC1*—a gene suspected of playing a role in breast cancer—had a functional effect. Nakamura, Kaufmann, Swenberg, and epidemiologist Bob Millikan applied this finding to their work with human cell samples from the Carolina Breast Cancer study, which is led by Millikan. Early findings suggest that this polymorphism in *XRCC1* increases the risk of breast cancer. Swenberg stresses that these are preliminary results, but that "it's an example of how bringing together epidemiologists, basic researchers, and clinicians can lead to new discoveries."

Exposure in the Most Vulnerable

Researchers in the Developmental Susceptibility Research Core examine the effects of exposures during the most vulnerable periods of human development: studies are investigating how environmental exposures from preconception through childhood contribute to such effects as miscarriage, birth defects, developmental deficits such as autism, and childhood diseases such as cancer. "The collaborations facilitated by the center make possible many new projects," says core director Andrew Olshan, a professor of epidemiology.

Smoking guns? Center researchers are investigating the interplay of genetic susceptibility and environmental exposures in causing disease.

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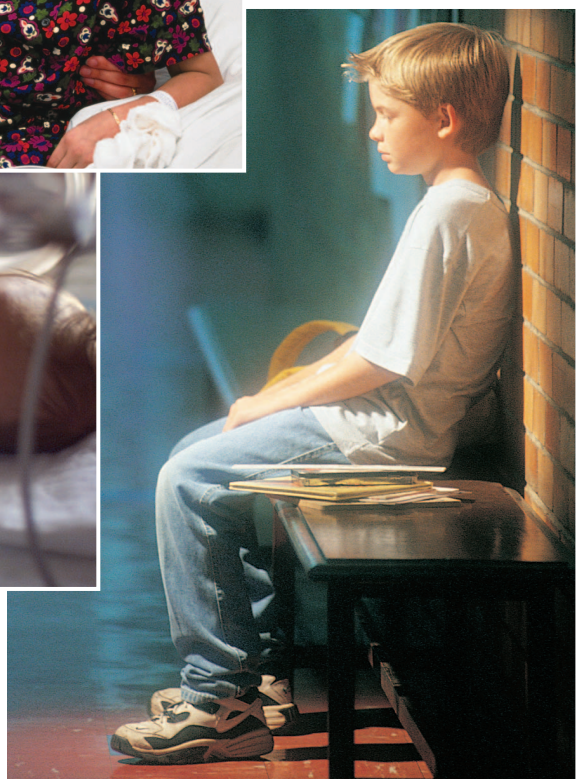
Core accomplishments include findings from nutritionist Steven Zeisel, published in the 12 March 1999 issue of *Developmental Brain Research*, that show that inadequate choline (a nutrient found in milk and eggs) in the diet during pregnancy alters fetal brain biochemistry, with resulting deficits in memory and mental processes that last throughout the offspring's lifetime. In the March–April 2001 issue of *Developmental Neuroscience*, Zeisel and colleagues described how choline supplementation during pregnancy influences development of the hippocampus—which governs learning and memory, among other functions—by altering the timing of the creation and differentiation of progenitor cells known to be associated with these functions in the adult brain. In unpublished research, Zeisel has also shown that diethanolamine (a chemical used in shampoos, lotions, creams, and other cosmetics) can induce choline deficiency in mice. He is doing further research to determine whether frequent use of this compound causes abnormal brain development in offspring of exposed mice.

In other core work, Stephanie Mulherin Engel, now a postdoctoral fellow at the Mount Sinai School of Medicine, studied the role that inflammatory cytokines (messenger chemicals produced inside the body in response to infections and toxicants) play in preterm and small-for-gestational-age births. For her doctoral dissertation, Engel worked under the direction of Olshan and collaborated with David Savitz of UNC-CH and Stephen Chanock, an investigator at the National Cancer Institute, to confirm that some known combinations of polymorphisms in cytokines increase the risk of spontaneous preterm birth; the team also identified others that had not been previously studied.



Knowledge growing by leaps and bounds. Center research is leading to a better understanding of the ways in which environmental exposures from preconception throughout childhood can influence outcomes including preterm birth, childhood cancer, and autism.

"Preterm birth is one of the most important contributors to infant mortality, but the causes are unknown," Olshan says. "This work gives us more information about the inflammation pathway that is suspected to affect preterm birth and about the genetic variants that may, in combination with exposures, affect a woman's risk of preterm birth." Savitz, Mulherin, and Olshan, along with obstetrics and gynecology professor John Thorp, recently submitted an NIH grant proposal to further examine the impact of exposures, genetic variation, and other factors on cytokine levels in pregnancy, especially among African Americans.

Investigators in the Toxicokinetic Susceptibility Research Core study the mechanisms of how toxicants found in air, water, and food are processed by the body, and how toxicants cause greater effects in some people than in others. Stephen Rappaport, a professor of environmental science and engineering, directs this core.

One high-profile example of this core's work is the ongoing Long Island Breast Cancer Study Project, one of the largest environmental epidemiological studies conducted on breast cancer. Led by CEHS deputy director Marilie Gammon, researchers examined 1,000 blood samples from newly diagnosed breast cancer patients and controls. The study focused on biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs), which are by-products of combustion found in air pollution, cigarette smoke, cooked foods, and other sources. These markers, called PAH–DNA adducts, are alterations to DNA that are considered precursor lesions to cancer. One report from this study, published in the August 2002 issue of *Cancer Epidemiology Biomarkers & Prevention*, showed that women with the highest quartile of PAH–DNA adducts had a 50% increased risk of breast cancer.

But the study did not find a simple cause–effect relationship. Subjects with the highest levels of exposure to PAHs did not necessarily have the most PAH–DNA adducts or the highest risk of breast cancer. Gammon says, "Our data indicate that PAH–DNA adduct formation may influence breast cancer development, although the association does not appear

Clockwise from top: Photodisc; Digital Vision; Getty Images

to be dose-dependent and may have a threshold effect. The risk for breast cancer in relation to PAH–DNA adducts did not vary when we examined whether someone was a smoker or nonsmoker, or when the subject reported eating grilled and smoked foods or not. So you can take two people with the same exposure levels, and one gets adducts, the other doesn't." Recent findings based on data from more than 1,800 women confirm these results; this report will appear in an upcoming issue of *Archives of Environmental Health*. The team is continuing to study these issues, focusing on the idea that individuals have different responses to environmental exposures because of their genetic makeup or other factors.

Fighting fat. The center's Obesity Research Core seeks to get at the heart of why some people weigh too much and how obesity predisposes people to a myriad of other health problems.

The center's second new research effort, the Obesity Research Core, directed by associate professor of nutrition and medicine Joyce Harp, includes researchers from nutrition, medicine, pediatrics, epidemiology, surgery, and other fields who will study how the environment impacts the development and maintenance of obesity. For instance, many compounds such as the pesticide DDT are lipophilic (easily dissolved and stored in fat). When someone who is obese loses a large amount of fat, such as in the increasingly common gastric-bypass surgery, what happens to the lipophilic compounds?

"Do these chemicals just redistribute into the remaining fat? That is what we hypothesize," Swenberg says. "Do the levels of environmental toxicants triple or quadruple if someone loses [hundreds of pounds]? We don't know. We'd like to find out something about the toxicokinetics, . . . exactly what happens." In addition to Swenberg, other core members involved in these studies include Zeisel, lipid biochemist Rosalind Coleman, and surgeon Timothy Farrell.

Other researchers will study obesity-related alterations to the immune system that may affect a person's response to infection, cancer susceptibility, and other characteristics. Researchers also are investigating the environmental factors that contribute to obesity, including nutrition and the built environment—the density and proximity of recreation facilities, transportation options, and pedestrian walkways.

Dispelling Genetic Myths

Drawing on the center's extensive research in breast cancer and other conditions, staff of the COEP, directed by environmental sciences and engineering professor Frances Lynn, have conducted workshops and other outreach programs throughout North Carolina. "Center scientists are intimately involved in all the programs we design for the public," Lynn says. Many of the programs help people better understand how their genes and their environment interact to affect their disease risk. For instance, Lynn obtained a supplemental grant from the NIEHS to develop materials on gene–environment interactions and ethical dilemmas posed by genetic testing (such as employment discrimination based on a person's genetic data and the psychological impacts of genetic testing).

Through conducting workshops across the state, Lynn found that many women believe that inherited mutations in genes play a large role in explaining the occurrence of breast cancer. Lynn and her staff draw on CEHS research by Gammon, Millikan, and others to dispel that myth. "Only five to ten percent of breast cancers are caused by a woman's inherited genetics, and an even smaller percentage is thought to be explained by *BRCA1* and *BRCA2*, the so-called breast cancer genes," Lynn says.

COEP staff tailor programs to various audiences. Senior citizen groups have responded well to "Reduce Breast Cancer Bingo," in which players fill their cards by answering questions about the role that various factors such as obesity, alcohol consumption, and exposure to PAHs in air pollution and environmental cigarette smoke play in breast cancer risk. With other groups who are interested in more scientific detail, participants read and discuss case studies of three fictitious women who show various risk factors for breast cancer and the ethical dilemmas that genetic testing can raise.

In addition, the COEP has developed a middle- and high-school science curriculum, "Our Genes, Our Environment, Our Health," and conducted workshops with 78 science teachers and 85 students in North Carolina. The curriculum complies with the North Carolina Standard Course of Study and includes activities in which students focus on ethical issues.

COEP science educator Michele Kloda stresses the importance of sharing the center's research with students and teachers: "North Carolina teachers are required to teach students the science behind gene–environment interactions, but very often the information they have access to is out of date or unrelated to students' lives. In contrast, we can provide teachers with current center research and activities about heart disease, tobacco smoke exposures, and obesity, and ultimately make environmental health science more relevant and accessible to young adults."

Canine contributions. Researchers hope that studies of dogs in Mexico will yield clues about how air pollution may contribute to neurodegenerative disease.

Investing in the Future

The center places great emphasis on fostering young investigators, providing junior investigators with formal grant-writing instruction and targeting its pilot project funding program toward them. Swenberg hopes this emphasis will mean more

Top to bottom: Getty Images; Jeffry Scott

future growth for the center and for environmental health science as a whole. "We feel that bringing junior investigators into environmental health research is a very critical responsibility of the center," he says.

Those efforts do seem to be paying off. In fiscal years 2001 and 2002, recipients used $165,000 in CEHS pilot funds to garner a 22-fold return of more than $3.6 million in external awards. CEHS members are directing eight nationally competed training grants, three of which are funded by the NIEHS.

Lilian Calderón-Garcidueñas, a recent Ph.D. recipient in toxicology and an advanced postdoctoral fellow in Swenberg's lab, is a recent example of these successes. She used a CEHS pilot project grant to begin research that suggests that severe air pollution may contribute to neurodegenerative disease. In the May 2002 issue of *Toxicologic Pathology*, Calderón-Garcidueñas and colleagues report on their study of dogs in Mexico City, which is known for its severe air pollution. Their results indicate that otherwise-healthy lifelong canine residents show evidence of changes in the frontal cortex, hippocampus, and olfactory bulb, compared to dogs from a nonpolluted rural area. These changes include upregulation of some inflammatory cytokines and genes such as COX-2, which is related to inflammation, and increases in amyloid-beta, which is a precursor lesion of Alzheimer disease.

Calderón-Garcidueñas will study this further by looking for similar changes in human brains and by investigating how the changes occur. In collaboration with CEHS epidemiologists, she will use geographic information systems to look at the distribution of cases to find out what types of pollution may be more likely to cause neurodegenerative damage. In a separate unpublished study, Calderón-Garcidueñas and colleagues found that air pollution may be related to chronic pulmonary hypertension; among 48 children who resided in Mexico City, 29% showed evidence of high pulmonary pressure. The epidemiological impact of this finding in a city where 6 million children live needs to be addressed, says Calderón-Garcidueñas.

"These findings have huge implications for environmental health," Swenberg says. "And this entire research program was funded out of a pilot project from our center. We think that encouraging young people to go into environmental health research and helping them have success during their early years of research is very likely to make them lifelong environmental health researchers. **–Angela Spivey**

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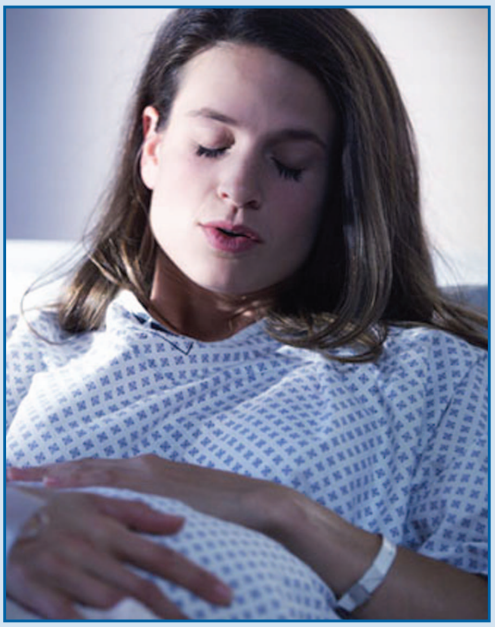
Headliners
NIEHS-Supported Research

Developmental Neurotoxicity



Drug Used to Arrest Preterm Labor Sensitizes the Brain to Neurotoxicants

Rhodes MC, Seidler FJ, Qiao D, Tate CA, Cousins MM, Slotkin TA. 2004. Does pharmacotherapy for preterm labor sensitize the developing brain to environmental neurotoxicants? Cellular and synaptic effects of sequential exposure to terbutaline and chlorpyrifos in neonatal rats. Toxicol Appl Pharmacol 195:203–217.

A growing body of evidence suggests that exposure to environmental toxicants *in utero* or very early after birth can have life-long effects. This phenomenon is referred to as the fetal basis of adult disease. Hypertension, diabetes mellitus, asthma, and cardiovascular diseases are but a few of the illnesses that have been suggested as possible effects from early-life exposures. Recently, NIEHS grantee Theodore A. Slotkin of Duke University Medical Center and his colleagues investigated how separate and combined exposures to terbutaline, a drug used to arrest preterm labor, and to the organophosphate pesticide chlorpyrifos affect several indices of brain cell growth and function.

Premature labor occurs in approximately 20% of all U.S. pregnancies, with preterm delivery—a leading cause of neonatal morbidity and mortality—occurring in nearly half these cases. Drugs to arrest preterm labor, particularly terbutaline, are used in as many as 1 million pregnancies annually. Chlorpyrifos, a known developmental neurotoxicant, is used worldwide.

Rat pups were given terbutaline on days 2–5 after birth, followed by chlorpyrifos on days 11–14. Neither treatment affected the growth or viability of the young rats; however, both elicited alterations in brain cell differentiation and cholinergic innervation at day 15, persisting into adulthood at day 60. Biomarkers of brain cell number, cell size, and neuritic projections were affected by both agents alone. However, the combined exposure produced more severe effects by both additive and synergistic mechanisms.

These findings suggest that terbutaline, like chlorpyrifos, is a developmental neurotoxicant. The authors conclude that the use of terbutaline to prevent preterm labor may be creating a subpopulation that is more sensitive to the adverse neural effects of organophosphate pesticides. Further studies are needed to repeat these findings, but if the results are confirmed, use of these compounds may warrant additional scrutiny. **–Jerry Phelps**

And the Oscar Goes To...

beyond the BENCH

Shakespeare said, "All the world's a stage." At the NIEHS Center in Environmental Toxicology at the University of Texas Medical Branch (UTMB) in Galveston, they're taking that concept a step further: two new programs within the center's Community Outreach and Education Program (COEP) strive to educate people about environmental health problems in the world around them through the experience of theater. The Translational Theater Outreach and Education (T-TOE) division translates the center's research science into plays, skits, and other forms of theater to raise awareness of health issues while providing a creative and interactive learning experience that empowers children and adults to adopt preventive behaviors to enhance wellness. The Public Forum and Toxics Assistance (PFTA) division uses Community Environmental Forum Theater productions to develop public awareness of linkages between toxic exposures and human health, and to promote dialogue on risk assessment and public environmental policy.

Young scientists get in on the act. T-TOE staff use theater to teach students at Houston's West University Elementary about the health effects of ground-level ozone.

T-TOE projects serve Galveston and Harris Counties directly, and provide professional development to early education and primary school teachers across Texas. The main component of the program is the Theater Troupe, a collaboration of center scientists, teachers, and artists that develops and performs original theater productions about environmental health issues such as asthma, vaccines, lead poisoning, pesticides, and pollution for students in kindergarten through twelfth grade. In fall 2004, two productions will tour Houston and Galveston in collaboration with Houston's John P. McGovern Museum of Health and Medical Science.

T-TOE productions move into the classroom through the "Positive Drama in the Classroom" module offered through the NIEHS Summer Teacher Training Institute, which is jointly sponsored by the NIEHS centers at the University of Texas M. D. Anderson Cancer Center and UTMB. The module is composed of activities that encourage exploration of environmental science through music, art, dance, and theater. These activities aid in developing a myriad of life-essential skills, including the enhancement of cognitive skills, engaging in creative and critical thinking, exploring kinesthetic intelligence, improving oral language skills and reading comprehension, identifying causal elements in thematic games and exercises, and strengthening confidence.

Another interactive classroom module, "Ozone Theater," was created in collaboration with the Houston nonprofit group Mothers for Clean Air. This module focuses on the health impacts of excessive surface ozone and positive actions that students can take to lead healthy lives. The module is designed for use by volunteer para-educators or professional teaching artists within a classroom setting.

The PFTA program uses drama in collaboration with communities that experience toxic exposures or desire a deeper understanding of environmental hazards. In communities from Corpus Christi to Port Arthur, the PFTA has offered Community Environmental Forum Theater workshops based on the "image theater" and "forum theater" techniques and formats developed by Brazilian playwright Augusto Boal. In Boal's innovative formats, audience members play characters in scenes and improvise new solutions to the community problems being presented.

These Community Environmental Forum Theater workshops create realistic dramas that show how environmental health issues affect everyday life in communities. They also encourage honest dialogue and informational exchange on basic toxicology, risk assessment, exposure levels and pathways, biomarkers, bioaccumulation and bioavailability, body burdens, toxicogenomics, and the distribution of disease susceptibilities within human populations. These dramas offer citizens, scientists, health care providers, environmental regulatory officials, and local governments an opportunity to realistically appraise risks and set goals for a healthier environmental future. The PFTA also offers a workshop in adapting forum theater techniques for use in environmental science classrooms through the NIEHS Summer Teacher Training Institute.

The learning never stops. PFTA staff and members of the Corpus Christi group Citizens for Environmental Justice act out community reactions to a suspected cluster of congenital heart disease.

The PFTA recently trained a bilingual *teatro* of community para-educators and actors—De Madres à Madres Promotores de Salud—to use forum theater as a primary outreach for the center's Project COAL (Communities Organized Against Asthma and Lead). Project COAL unites the NIEHS center with De Madres à Madres, a North Houston community-based organization supporting at-risk pregnant Hispanic women, and Casa de Amigos, a Harris County primary health care provider, in a community-wide effort to decrease childhood lead poisoning and residential asthma triggers. This program deploys the *teatro* as a way to assess needs, disseminate information, and evaluate results throughout the project. The PFTA also collaborates with Frontera de Salud, a health organization run by student health care providers, and UTMB's Stark Diabetes Center in bringing bilingual diabetes management dramas to the impoverished Cameron Park community in Brownsville. **–Travis J. Mader**

Travis J. Mader